

Developmental and Behavioral Changes in the Rat during Chronic Exposure to Lead

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Young male and female Sprague-Dawley rats were given drinking water containing 5 or 50 ppm Pb for 40 days prior to mating. Pregnant females were continued on these regimens throughout gestation and lactation. After weaning the offspring were similarly exposed through adulthood. Reflex development, body weights, and locomotor activity were measured in the offspring. Significant delays were noted in the development of the righting reflex at 5 and 50 ppm and in eye opening at 50 ppm. No difference was observed in development of the startle reflex at either dose. Mean body weights of treatment groups during this developmental period were not significantly different from controls. Locomotor activity was measured in adult males utilizing a residential maze. Both levels of lead produced a significant reduction in locomotor activity. When groups were treated with *d*-amphetamine (4.0 mg/kg subcutaneous), lead treatment caused a dose-related diminution in the amphetamine-induced hyperactivity.

These results indicate that rats exposed to low levels of lead from conception until adulthood show a delay in nervous system development. As adults, these animals exhibit hypoactivity and decreased responsiveness to amphetamine.

Introduction

Lead toxicosis in children has been implicated in hyperkinesia (1) and mental retardation (2). These findings have raised the possibility that chronic, low-level exposure to lead may produce "silent" brain damage. Animal experiments, using a variety of species, have demonstrated that perinatal exposure to lead produces similar behavioral changes including hyperactivity, peripheral ataxia, and aggressiveness (3-5). These experiments, however, have employed dietary levels of lead ranging from 1200 to 27,000 ppm.

The present study was undertaken to test for possible developmental and behavioral changes produced by continuous pre- and postnatal exposure to low levels of lead. The results indicate that behavioral changes can be produced by levels as low as 5 ppm in the diet.

Methods

Male and female Sprague-Dawley rats, obtained from Blue Spruce Farms (Altamont, N.Y.), were randomly assigned to one of three treatment groups: 0, 5, or 50 ppm lead administered in the drinking water in the acetate form. Animals were pretreated for a period of 40 days, which allowed lead concentrations to approach steady-state levels in soft tissues (6). Following pretreatment, animals were mated and pregnant females were continued on lead throughout gestation and lactation. At weaning, the offspring were similarly exposed through 180 days. These F₁ generation rats were mated at 90 days of age, and the resulting F₂ generation animals were exposed to the same lead regimen.

Five pups from each of 4-6 litters per treatment group in both the F₁ and F₂ generations were tested for the ages at development of the startle response, the righting reflex and the age of eye opening. The startle response was evoked by a click (generated by a toy clicker) sounded immediately behind the

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head of an animal held suspended by the nape of the neck. A positive response consisted of a muscle jerk in any of the extremities.

The righting reflex was elicited by holding the rat upside down by the nape of the neck and the tail and dropping it from approximately 30 cm above some wood shavings. A response was considered positive if the animal righted in mid-air and landed on all four feet.

Age at eye opening was recorded with complete opening of both eyes.

Five pups from each of 9–11 litters ($F_1 + F_2$) per treatment group were tested daily between 8:30 A.M. and 10:30 A.M. The litter means were used to calculate the mean day of development of each response, for each treatment group.

Locomotor activity of adult males was recorded at approximately 120 days of age in a residential maze (Fig. 1) described initially by Norton et al. (7). Groups of three littermates were tested in the maze for a period of 5 days. Four mazes were housed in a sound-attenuated room maintained on a 12 hr light/12 hr dark cycle beginning at 6:00 A.M. Food and water were available *ad libitum*. The daily experimental procedure was as follows: Groups were introduced into the mazes at 10:00 A.M. and removed at 9:00 A.M. the following day, the mazes were cleaned, food and water were replenished, and the animals were then reintroduced into the mazes at 10:00 A.M. Activity counts were recorded at 1-hr intervals by utilizing a Xerox minicomputer.

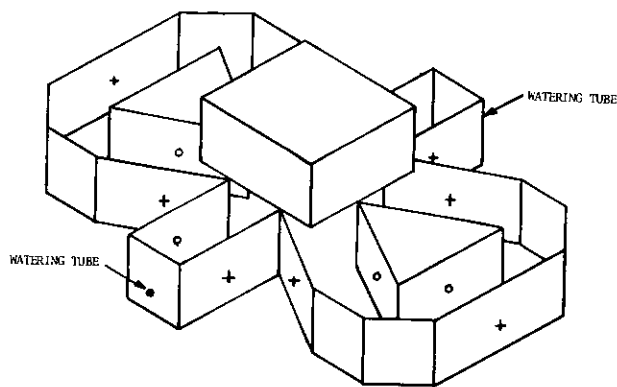


FIGURE 1. Schematic representation of the residential maze used to measure locomotor activity. The maze consists of a series of interconnected alleys (10 cm wide, 10 cm high) converging on a central openfield and covered with transparent acrylic plastic equipped with a central raised area over the open field. The overall dimensions are 76 cm wide x 60 cm deep. Locomotion is detected by eight phototransistor (+)/infrared light-emitting diode (o) pairs.

Maze activity was also recorded following the administration of *d*-amphetamine HCl (4 mg/kg) administered subcutaneously (SC) on day 5, 20 min prior to reintroducing the animals into the maze.

Group means, for both reflex development and locomotor activity, were compared by using the *t*-test.

Results

No differences between the F_1 and F_2 generations were observed in any parameter and results for both generations have been combined.

The effects of lead treatment on development are shown in Table 1. Development of the startle response, which occurs normally at 12 days of age, was not significantly altered by lead treatment. On the other hand, both eye opening and development of the righting reflex were significantly delayed ($p < 0.05$) by lead administration. Eye opening was delayed approximately 1 day by the 50 ppm treatment, whereas the righting reflex was delayed 1 day by 5 ppm and 2 days by 50 ppm.

Table 1. Effects of lead administration on reflex development and eye opening in the rat.

Pb dose, ppm ^a	No. of litters N ^b	Mean day of development \pm S.E.		
		Startle	Eye opening	Righting
0	11	11.8 \pm 0.2	15.2 \pm 0.2	17.7 \pm 0.2
5	9	12.0 \pm 0.2	15.4 \pm 0.2	18.8 \pm 0.4 ^c
50	10	12.4 \pm 0.2	15.8 \pm 0.2 ^c	19.8 \pm 0.7 ^c

^aDose given as ppm of lead in the drinking water.

^bN = number of litters (5 animals/litter)

^cSignificantly different from control, *t*-test, $p < 0.05$.

Body weights for male rats during this period of developmental testing are shown in Figure 2. No consistent differences in body weights of the treatment groups were observed, although the 50 ppm lead group was significantly heavier than controls on days 15 and 18. Females showed similar growth curves.

Figure 3 shows representative 23-hr time-interval histograms for the various treatment groups taken on day 3 in the maze.

Animals showed an initial high level of exploratory activity during the first hour in the maze. Following this initial period of exploration, activity showed a normal circadian rhythm with low diurnal (photolight) activity and high nocturnal (photodark) activity. Data obtained during these various periods, i.e., exploratory, diurnal, and nocturnal as well as total activity were analyzed for lead effects.

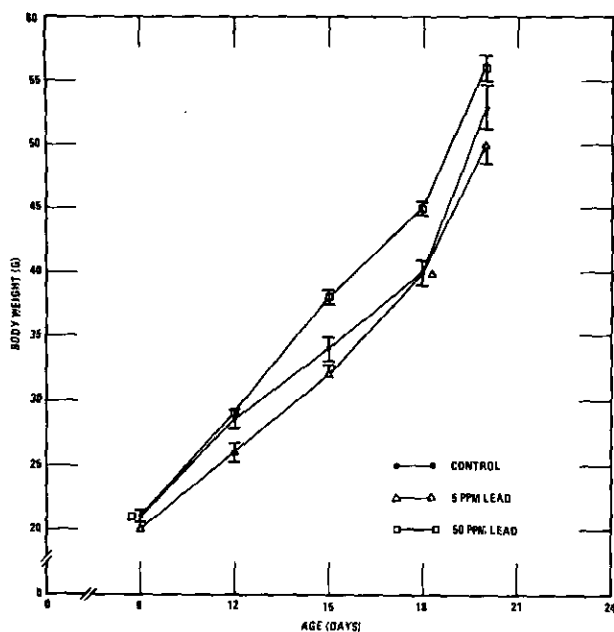


FIGURE 2. Growth curves for control and lead-treated male rats during the period of developmental testing: (●) control; (Δ) 5 ppm Pb; (□) 50 ppm Pb. Each point represents the mean (\pm S.E.) for 12 animals.

The compiled data for maze activity on the first four successive days of testing are presented in Figure 4. Activity on the first day in the maze was higher than on subsequent days. This difference was observed in all treatment groups and during all time periods. This elevated activity represents the animal's increased reactivity due to the novel environment. By the second day, the animals became "established" in the maze and subsequent activity levels were consistent from day to day.

Lead administration produced a significant ($p < 0.05$) decrease in locomotor activity ranging from 27 to 42% on day 1 and from 11 to 37% on day 4. This lead-induced hypoactivity was not dose-related. Both total and nocturnal activity remained depressed in both lead-treated groups throughout the 4-day period. By day 4, neither lead-treated group differed from control during the exploratory period and following the first day no group differences were found in the diurnal period.

Twenty minutes prior to testing on day 5, animals were given subcutaneous (SC) injections of 4.0 mg/kg *d*-amphetamine HCl. The results presented in Figure 5 represent the change in activity (Δ) following amphetamine administration. The Δ activity was determined by subtracting the activity obtained on day 4 (predrug, control levels) from the

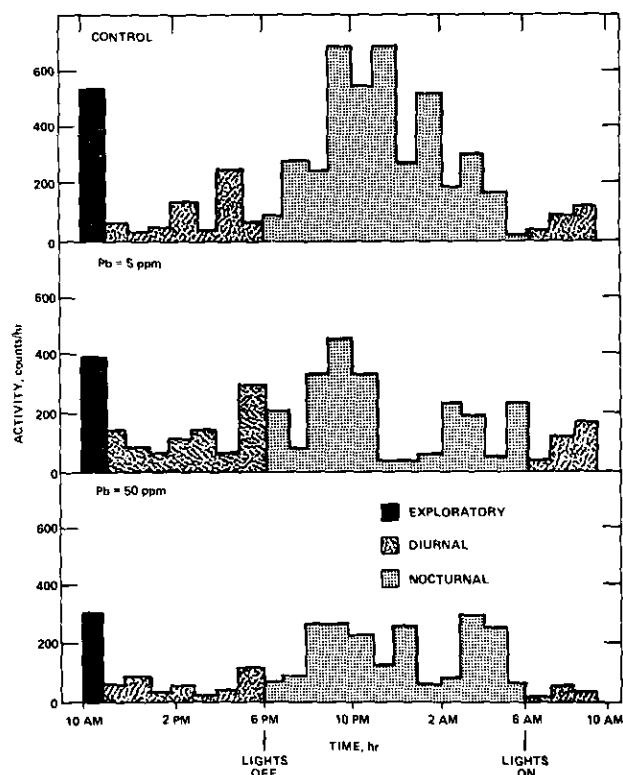


FIGURE 3. Representative time-interval histograms from control and lead-treated animals. Data was obtained on day 3 in the maze from groups of three adult males. For purpose of analysis, the data has been divided into the various activity periods represented by the shaded areas.

activity following amphetamine and therefore represents the drug-induced changes in activity.

In control animals, amphetamine produced the expected hyperactivity. Since drug was administered during the diurnal period, when activity is normally low, the major effect was seen during this period. Activity in control animals was elevated to 444% and 682% of predrug levels, during the exploratory and diurnal periods, respectively. This was also reflected in an elevation of total activity to 224% of predrug control level. During the nocturnal period, however, the control activity fell to 80% of the predrug level, demonstrating a partial compensation for the drug-induced hyperactivity.

Lead-treated animals showed a diminution in the amphetamine response (Fig. 5). When compared to the Δ activity of the control group, both treatment groups showed a significant ($p < 0.05$) decrease in amphetamine-induced activity. This decreased responsiveness was also observed when activity was expressed as percentage of predrug

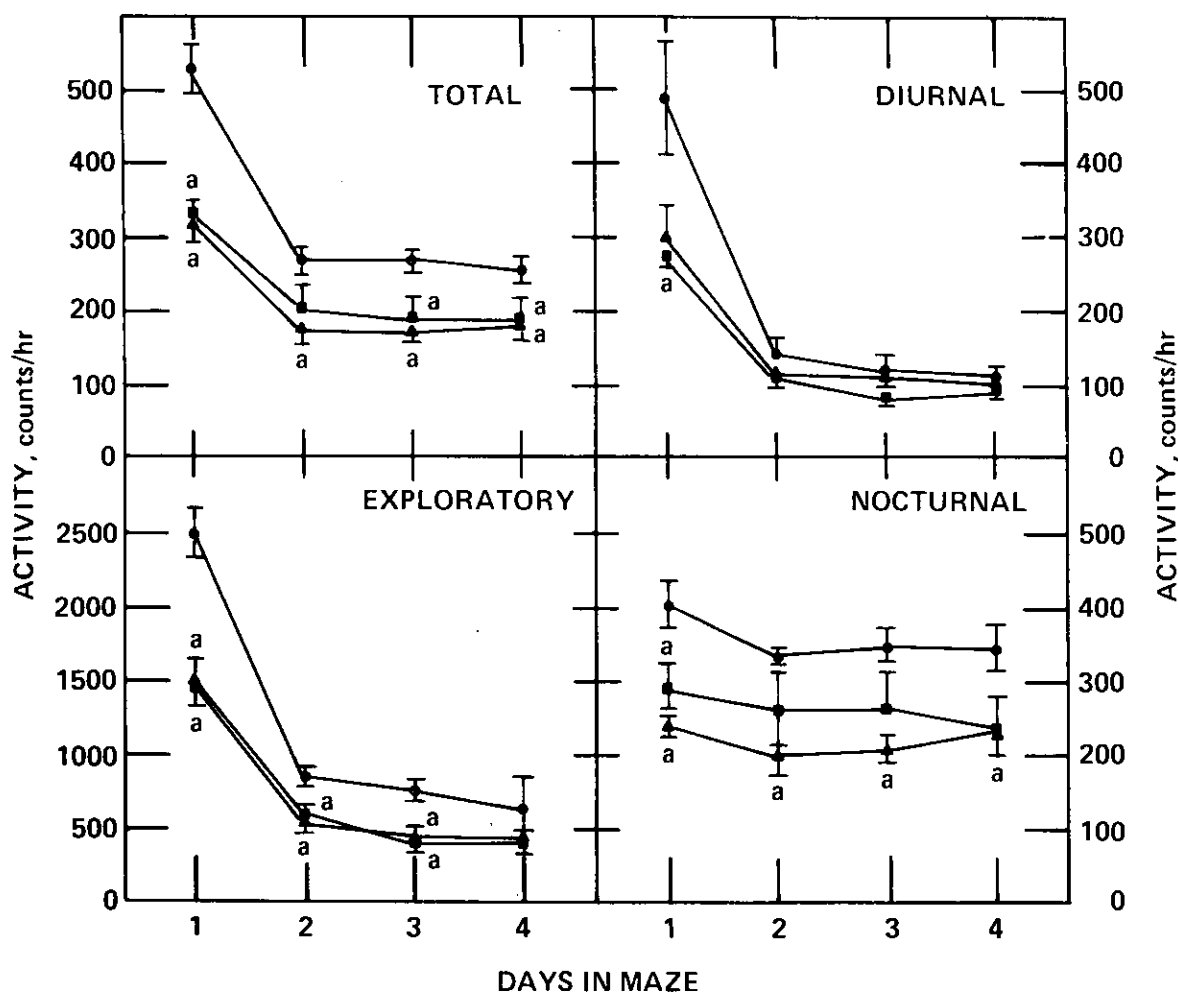


FIGURE 4. Effects of lead administration on locomotor activity in adult male rats: (●) control; (Δ) 5 ppm Pb; (□) 50 ppm Pb. Animals were tested in groups of three, and each point represents the mean (counts/hr + S.E.) for five groups of animals. Data are presented for the various activity periods for the first 4 days in the maze. Points marked "a" are significantly different from control (*t*-test, *p* < 0.05).

levels. Whereas control activity was elevated to 224, 444, and 682% during the total, exploratory, and diurnal periods, respectively, the 50 ppm group was elevated to 168, 323, and 398% during these same periods. As with the controls, treatment groups showed less activity during the nocturnal period and no significant differences between groups were present during this period.

Discussion

Rats continuously exposed from conception to low levels of lead showed a delay in nervous system development as seen by a delay in the righting reflex. Perinatal exposure has previously been

reported to cause delayed development (8), but at dietary levels which produced a fall in food consumption with a concomitant retardation of growth. This undernutrition, which is known to delay development (9), was sufficient to produce a similar delay in pair-fed controls. In the present study, no differences in growth rates were observed during the period of reflex testing which suggests that the observed delay in development was the result of a direct effect of lead on the nervous system of the pups.

Daily locomotor activity was depressed during four consecutive days of testing in adult lead-treated animals. On the first test day, hypoactivity was observed during all periods of the daily activity cycle. However, on subsequent days no differences

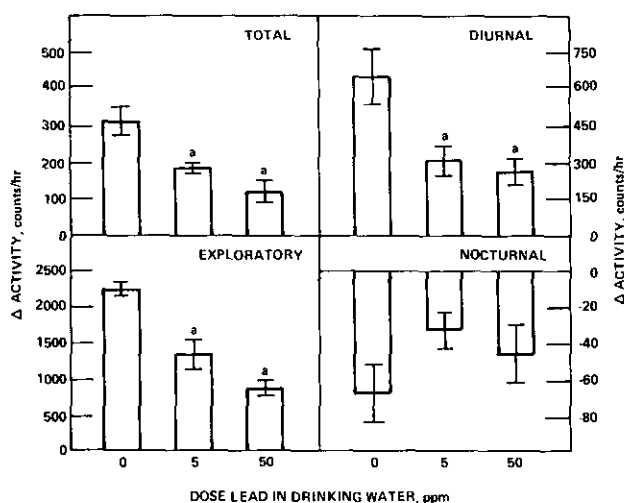


FIGURE 5. Effects of *d*-amphetamine administration (4.0 mg/kg) on locomotor activity of control and lead treated rats. Values are presented as Δ activity (counts/hr \pm S.E.) following amphetamine; Δ activity = (activity on drug day) - (activity on the predrug day). Each bar, therefore, represents the drug-induced locomotor activity for five groups of animals. Bars marked "a" are significantly different from control (*t*-test, *p* < 0.05).

were observed in diurnal activity and by the fourth day, exploratory activity (defined as the first hour in the maze) was also at control levels. This transient difference in activity suggests that lead treatment alters the animal's reactivity to a novel situation and that once stable activity is established, only nocturnal activity is affected by lead treatment. Similar variations in the responsiveness of animals to administration of other chemical agents as a function of time have been described (10).

This finding of hypoactivity has added more fuel to the fire of confusion which consumes the lead literature. All possible changes in locomotor activity have been reported following lead treatment: hyperactivity (4,11), hypoactivity (12), and finally, no change in activity (13).

In the present study, animals were tested as adults. The possibility exists that the lead effect on activity in the rat is age-dependent with initial hyperactivity in young animals (11) followed by hypoactivity in the adult. This time course would be consistent with the relationship of lead toxicosis with minimal brain dysfunction in children since the hyperactivity in children generally disappears with age, the so-called "maturational lag" (14). The serial time course of activity changes in lead

treated animals is now being tested in our laboratory.

Finally, the observed attenuation in the response of lead-treated animals to amphetamine administration has been consistently observed both in mice (15) and rats (13). Furthermore, this diminished responsiveness has been observed when baseline activity is elevated (15), normal (13) or depressed (present study) by lead treatment.

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